

# Gene expression profiling in the post-mortem human brain — no cause for dismay

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## Abstract

Global expression profiling techniques such as microarray technology promise to revolutionize biology. Soon it will be possible to investigate alterations at the transcript level of the entire human genome. There is great hope that these techniques will at last shed light on the pathological processes involved in complex neuropsychiatric disorders such as schizophrenia. These scientific advances in turn have re-kindled a great interest and demand for post-mortem brain tissue. Good quality post-mortem tissue undoubtedly is the fundamental prerequisite to investigate complex brain disorders with molecular profiling techniques. In this review we show that post-mortem brain tissue can yield good quality mRNA and intact protein antigens which allow the successful application of traditional molecular biology methods as well as novel profiling techniques. We also consider the use of laser-capture microdissection on post-mortem tissue. This recently developed technique allows the experimenter to explore the molecular basis of cellular function at the single cell level. The combination of laser-capture microdissection with high throughput profiling techniques offers opportunities to obtain precise genetic fingerprints of individual neurons allowing comparisons of normal and pathological states. © 2001 Elsevier Science B.V. All rights reserved.

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## 1. Introduction

With the completion of the human genome project, studies of gene expression in the normal and pathological human post-mortem brain are gaining momentum. For these studies to be successful it is imperative that intact mRNA can be extracted and analyzed. During the last decade concern and pessimism have been expressed about mRNA stability post-mortem. However, it is now clear that agonal status and the freezing of the tissue impact on mRNA integrity more than post-

mortem interval (Harrison et al., 1991, 1995; Kingsbury et al., 1995; Yates et al., 1990). Furthermore, with appropriate tissue preparation the structural integrity of post-mortem tissue can be preserved allowing for detailed morphological, morphometrical and ultrastructural investigations e.g. (Benes, 1988; Ravid et al., 1992; Vonsattel et al., 1995; Waldvogel et al., 1999).

Here we illustrate that indeed good quality mRNA can be extracted from post-mortem human brain tissue and that specific mRNA transcripts and gene products can be localized with in situ hybridization and immunohistochemical techniques, respectively. Furthermore, high-throughput mRNA expression profiling is possible using post-mortem human brain tissue and data from our laboratories demonstrate that robust and reproducible results can be achieved using an improved

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differential display technique (DD-PCR). Messenger RNA profiling studies are of particular value when applied to: (1) psychiatric and neurological disorders of unknown etiology; (2) the evaluation of cellular vulnerability in neurodegenerative disorders; (3) investigating the molecular basis of patient resistance to drug therapy; and (4) identifying the molecular basis of abnormal brain chemistry/function identified by sensitive *in vivo* neuroimaging techniques, for example SPECT, PET and fMRI. Post-mortem human brain material is a precious and valuable resource for molecular studies. An important factor in determining mRNA integrity i.e. full-length transcripts, is the processing and freezing of the brain post-mortem. Vonsattel and colleagues (1995) recently re-addressed this issue and have published detailed guidelines to assist brain banks and researchers in this quest. This protocol which requires the careful dissection and rapid freezing of tissue blocks in liquid nitrogen vapor is now closely adhered to by many brain banks, resulting in excellent quality tissue for molecular and ultrastructural studies. This protocol is a significant improvement over the traditional methods of passive freezing, which results in brain tissue suitable for biochemical, but not molecular, studies. Whether such passive-frozen tissue can be used for high-throughput protein profiling (proteomics) is unclear at present although studies from our laboratories and those of others have been able to use this material for immunohistochemical and ligand-binding studies. Given the current excitement about post-translational modifications of receptor proteins in neuro-degenerative disease (Dunah et al., 2000), these proteomic studies are eagerly awaited.

In addition to method of freezing, other caveats also exist when working with human post-mortem brain tissue and these are addressed below.

## 2. Human brain tissue

Two of the most critical factors in selecting tissue for inclusion in a study are neuropathological verification of the clinical diagnosis (if possible) and the exclusion of brains exhibiting co-existing pathology. This is especially critical for disorders such as Progressive Supranuclear Palsy (PSP) and Cortico-Basal Degeneration (CBD) where the final diagnosis is often made neuropathologically (Dickson, 1999). Furthermore, acute or distant brain lesions/infarcts or co-existing pathology such as Alzheimer's disease can confound the data analysis and interpretation. Thus, it is important to select post-mortem cases carefully, especially 'controls'. Where possible we routinely match 'normal' and 'disease' samples for several factors including age, gender, ethnicity, medications, ag-

onal state, post-mortem interval, disease severity, laterality of the brain and length of fixation (Benes, 1988). Strict adherence to these criteria can significantly aid in reducing inter-sample variation allowing for meaningful quantitative data to be obtained from relatively small ( $n = 4-6$ ) sample groups (Augood et al., 1996, 1997; Harrington et al., 1995a, 1996). More recently we have used genetics coupled with neuropathology to examine markers of metabolic dysfunction in the PSP brain (Albers et al., 1999, 2000). Haplotype analysis was used to confirm uniformity of the genetic background, whilst neuropathology was used to rule out coexisting AD pathology, as this would have confounded data interpretation. Clearly, such rigorous genetic and pathological markers are currently not available for the majority of neuropsychiatric disorders, here one has to rely on rigorous clinical diagnosis.

Once these criteria have been satisfied the next step is to extract and evaluate RNA integrity.

## 3. mRNA stability

Good quality mRNA is an essential prerequisite for the application of all molecular techniques including *in situ* hybridization, Northern blot analysis, RT-PCR and high throughput mRNA profiling. The belief that post-mortem delay critically influences mRNA stability is still a widely held notion despite overwhelming evidence that mRNAs are stable for long periods, at least up to 48 h (Barton et al., 1993; Harrison et al., 1997; Leonard et al., 1993; Schramm et al., 1999). More important than the interval is the storage temperature of the body in the post-mortem period. It is of note that ante- and peri-mortem factors, especially hypoxia, exert the most profound effect. Several studies have demonstrated that hypoxia due to prolonged agonal states significantly reduces mRNA and protein integrity and content (Harrison et al., 1991, 1995; Kingsbury et al., 1995; Yates et al., 1990). A simple and reliable, albeit crude, indicator of mRNA integrity is brain tissue pH (Kingsbury et al., 1995). Tissues with low pH ( $\text{pH} < 6.0$ ) have fragmented or absent mRNAs showing a strong correlation between tissue pH and mRNA quality. This correlation is consistent for both control and pathological brain tissue. Harrison et al. (1995) confirmed these findings demonstrating that in human and rat brain post-mortem interval had a limited impact on mRNA and protein integrity. Brain tissue pH can thus be used as a reliable indicator (superior to clinical assessment or mode of death and agonal state) of RNA quality; intact mRNA being associated with brain pH measurements in the range of pH 6.1–7.0. Furthermore, brain tissue pH is stable post-mortem, is unaffected

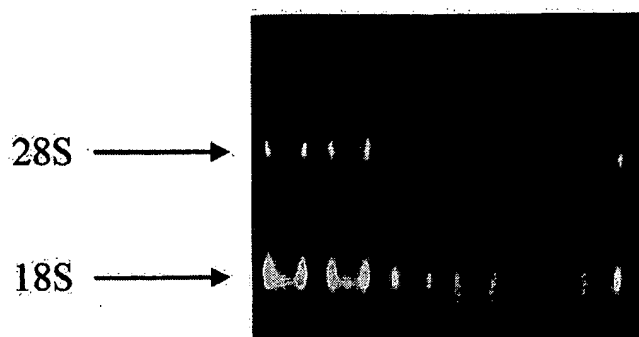


Fig. 1. Integrity of RNA extracted from human brain. Total RNA was prepared from post-mortem Alzheimer's and control prefrontal cortices.

during freezer storage and is remarkably consistent across different brain areas (Johnston et al., 1998) allowing the tissue pH of any region predict the mRNA integrity of all areas. Occasionally RNA can be extracted from tissues with low brain pH (pH 5.9–6.1) and samples can be used for selective RT-PCR based methods which do not require full length transcripts. This tissue is certainly sub-optimal and should be used with caution. For comparative mRNA profiling (cDNA microarrays or oligonucleotide GeneChips™) transcripts with intact 3' ends are required for first strand cDNA synthesis, hence brain tissues with intact mRNA should be used. Additionally, 0.1% acridine-orange histofluorescence can also be used to screen for DNA/RNA abundance in fresh frozen sections, although the inherent autofluores-

cence of post-mortem human brain can be problematic.

In our laboratories we routinely evaluate the purity and integrity of human brain RNA using a number of standard techniques.

### 3.1. Spectrophotometric assay

The purity and concentration of an extracted total RNA sample can be calculated by measuring the optical density (OD) of a sample aliquot at 260 and 280 nm, respectively. The  $OD_{260}/OD_{280}$  ratio should be in the range of 1.6–2.0 for pure RNA (Maniatis). We routinely extract total RNA from flash-frozen human post-mortem brain with an  $OD_{260}/OD_{280} = 1.7$ –1.8.

### 3.2. Visualization by gel electrophoresis

Total RNA integrity can be estimated by agarose gel electrophoresis. An aliquot of total RNA (approx 10 ug/well) can be loaded into a single well of a 0.8% agarose/MOPS gel and the RNA components separated electrophoretically. Staining of the gel with ethidium bromide should reveal the presence of two sharp bands at approx. 5 and 1.8 Kb corresponding to 28S and 18S ribosomal RNA (Fig. 1). Ethidium bromide smears as opposed to sharp bands indicates RNA degradation.

### 3.3. Reverse transcriptase PCR and cDNA synthesis

A more accurate indicator of mRNA integrity can be obtained by RT-PCR (Johnston et al., 1998; Schramm et al., 1999). So-called 'housekeeping genes'

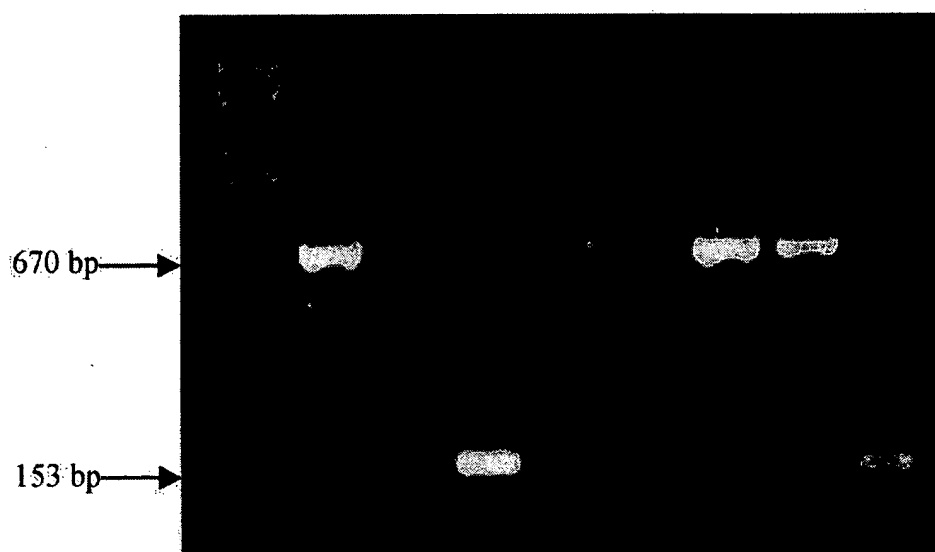


Fig. 2. Amplification of cDNA samples with calbindin-specific primers that detect genomic contamination by amplifying across intron/exon boundaries. Arrows illustrate cDNA (153 bp) and genomic contamination (670 bp).



Fig. 3. Macroscopic (A) and microscopic (B) localization of enkephalin mRNA within the caudate-putamen of a genetically- and pathologically-confirmed Huntington's disease case. Note the selective loss of enkephalin mRNA within the dorsal caudate nucleus and the 'patchy' distribution of hybridization signal within the ventral caudate and putamen (A).

such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and other known mRNAs of varying abundance and length can be amplified from first strand cDNA with mRNA-specific primers. Genomic DNA contamination of the RNA sample can be tested by using primers designed to amplify across exon/intron boundaries (Fig. 2).

#### 4. Localization of mRNA with in situ hybridization and Northern blot analysis

In situ hybridization (ISH) histochemistry is now a well-established technique that allows the qualitative and quantitative localization of specific mRNA transcripts at the macroscopic and microscopic level. Our laboratories have extensive experience with the application of these molecular techniques to human post-mortem brain tissue. When working with human post-mortem tissue it is critically important to include several controls in each hybridization step including the use of two or more oligonucleotide or cRNA probes complementary to non-overlapping sequences of the same mRNA. Furthermore, for quantitative Northern blot analysis it is important to normalize the mRNA abundance to an independent mRNA, thought not to be affected by the experimental design or disease process. This is currently a topic of active discussion and normalizing to 18S ribosomal RNA and/or housekeeping genes (e.g. GAPDH, actin) is still favoured. The most robust means of minimizing inter-group variability has been discussed above and include matching 'normal' and 'experimental/disease' group sizes.

Macroscopic and microscopic in situ hybridization are enormously powerful techniques for both the qualitative and quantitative assessment of mRNA abundance (Fig. 3). High-resolution microscopy of hybridized tissue sections processed for emulsion autoradiography can provide detailed information about heterogeneous cell populations (Fig. 3B). We and others have used these techniques extensively to examine the cellular expression of mRNAs encoding a number of dopamine receptors, glutamate receptors, serotonin receptors and neuropeptides in the post-mortem schizophrenic brain (Burnet et al., 1996; Eastwood et al., 2000; Eastwood and Harrison, 2000; Harrington et al., 1995b; Porter et al., 1997). Furthermore, our studies of early-grade Huntington's disease (HD) were amongst the first to identify a deficit in DA receptor expression within the HD caudate-putamen (Augood et al., 1997; Weeks et al., 1996). These findings are now being replicated and confirmed in various transgenic mouse models of HD (Bibb et al., 2000; Cha et al., 1999; Hodgson et al., 1999; Yamamoto et al., 2000) and are leading to new insights into the disease process (Chen et al., 2000). Other examples of recent studies of neurological diseases investigated using in situ hybridization and Northern blot analysis include Parkinson's disease (Fig. 4) (Eve et al., 1998; Harrington et al., 1996; Solano et al., 2000), Alzheimer's disease (Ross et al., 1992), PSP (Chambers et al., 1999), and DYT1 dystonia (Augood et al., 1998, 2000) (Fig. 5).

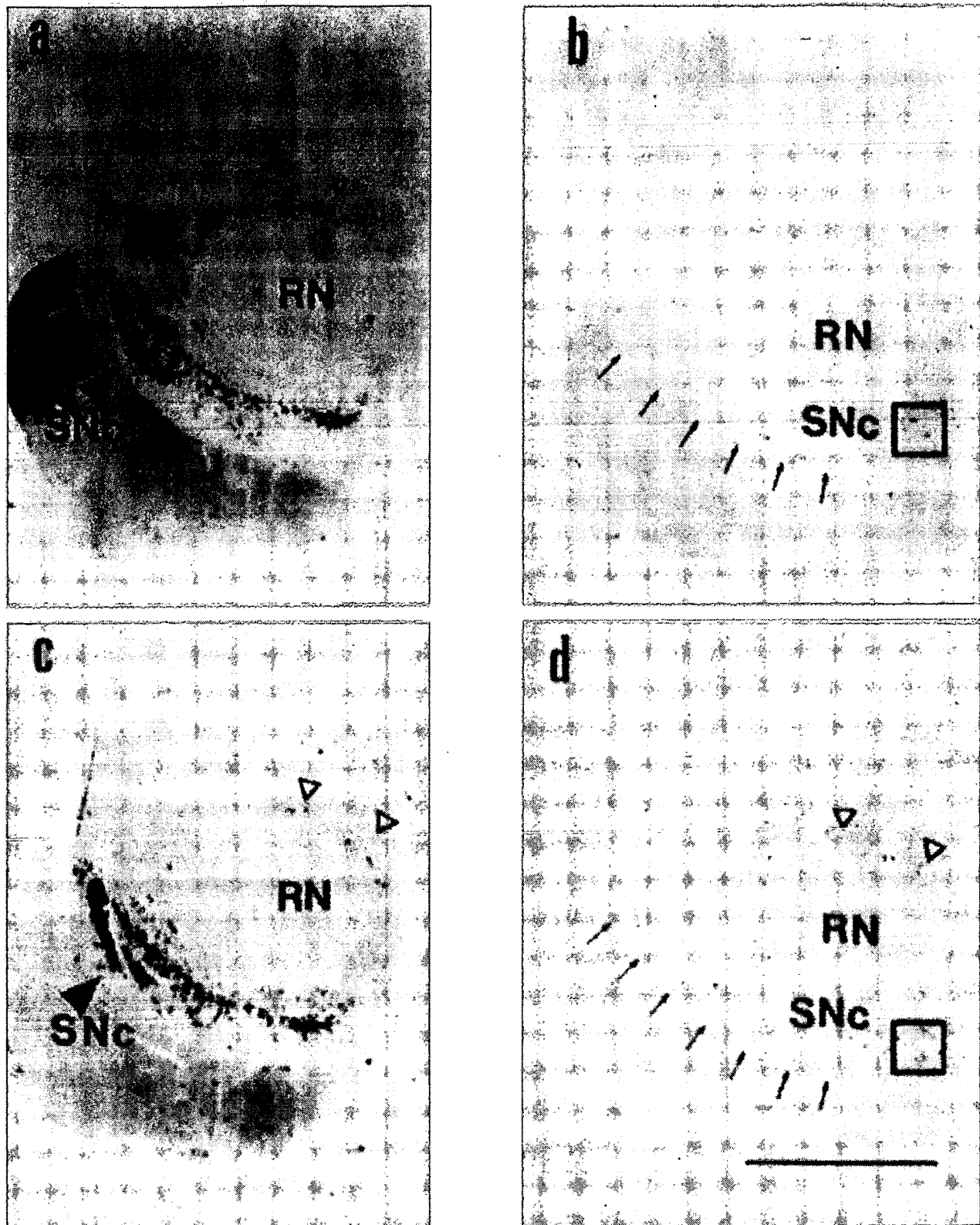


Fig. 4. Autoradiographic images of control (A,C) and Parkinson's disease (PD) (B,D) sections hybridised with either dopamine transporter (DAT) (A,B) or synaptic vesicle amine transporter 2 (VMAT2) (C,D) oligonucleotide probes. Note the marked loss of DAT and VMAT2 signal in the substantia nigra pars compacta (SNc: arrows) of PD nigra when compared to a control nigra. Some preservation of DAT signal is seen in the most medial aspects of the nucleus (boxed area). By contrast, individual VMAT2 mRNA-positive cells surrounding the red nucleus (RN) are detected in both control (C) and a PD (D) case (arrowheads). No obvious reduction in signal is observed in this region (Harrington et al., 1996).

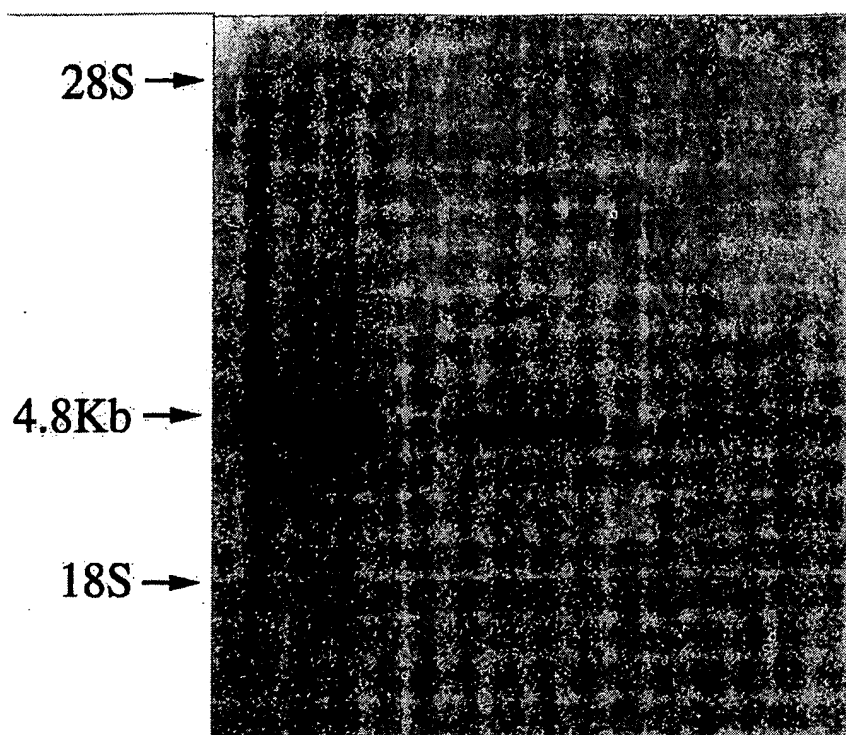


Fig. 5. Northern analysis of GAD67 mRNA in the frontal cortex from three different human brains. The nylon membrane was hybridized with a [ $^{32}$ P]-GAD67 cRNA probe. Note that a single band of approximately 4.8 Kb consistent is detected in all three samples, consistent with its predicted size. Positions of 18S and 28S ribosomal RNA are marked.

### 5. Localization of protein using immunohistochemical techniques

Immunohistochemistry using monoclonal and/or polyclonal antibodies is an established technique for localizing antigens within complex tissues. Indeed, we have most recently used this approach to map the regional distribution of a number of proteins including various G-protein coupled receptors and sodium channels within the human brain (Fig. 6) ([Moore et al., 2000 no. 4; Phillips et al., 2000 No. 5; Whitaker et al., 2000 No. 2) (Billinton et al., 2000 No. 3)]. We have also used immunohistochemistry to investigate the three-dimensional topography of the matrix/striosome compartmentation of the human caudate-putamen (Fig. 7) and to study the topography of calcium binding proteins within the human subthalamic nucleus (Augood et al., 2000). Furthermore, Benes and colleagues have used these techniques to demonstrate a 'mis-wiring' of neuronal circuitry within the schizophrenic brain (Benes and Berretta, 2000).

Semi-quantitative immunohistochemical data may be obtained if fluorescent or radioactively tagged secondary antibodies are employed. Classical immunoperoxidase methods can also be quantified if the reaction is topped in the linear range of the enzymatic reaction. For example, we have used quantitative immunohisto-

chemistry to demonstrate a significant reduction in substance P-immunoreactivity within the basal ganglia of parkinsonian primates (Augood et al., 1989). These findings directly complement our molecular studies showing mRNA specific alterations in neuropeptide expression within these parkinsonian primates (Herrero et al., 1995).

Generally immunohistochemistry and in situ hybridization complement each other with regard to data validation. However, as proteins are often trafficked to intracellular compartments external to the site of the perikarya, apparent discrepancies may be observed. For example, immunoreactivity for the neuropeptides enkephalin and substance P are enriched within the globus pallidus externus and internus, respectively, although the localization of their perikarya are within the caudate-putamen. Furthermore, apparent discrepancies can exist between mRNA abundance and the abundance/ultrastructural localization of the translated protein. For example, mRNA splicing or protein cleavage may result in truncated or lost epitope sites. Further, post-translational modifications of proteins can result in masked/modified binding sites, for example hyperphosphorylation of proteins induced by oxidative stress or agonist-induced phosphorylation and internalization of membrane-bound receptors.

## 6. Localization of proteins using receptor autoradiography

Receptor autoradiography is a robust technique for visualizing the regional localization of receptors in addition to the kinetics of receptor populations via  $K_d$  and  $B_{max}$  (Herkenham and Pert, 1982; Joyce et al., 1986; Penney and Young, 1982). Remarkably, receptor proteins are surprisingly stable post-mortem. However, levels of protein degradation should be critically assessed, for example by assessing receptor binding of a number of receptors which are not expected to be changed and can be used as an internal standard. In general, fresh frozen cryostat tissue sections are incu-

bated in the presence of a specific tritiated ligand, for example, [ $^3$ H]-SCH23390 for the localization of DA D1-like receptors (Fig. 8). Non-specific binding is defined in the presence of a selective displacer. After incubation of the tissue sections with the [ $^3$ H]-ligand, slides are rinsed in cold assay buffer and opposed to tritium-sensitive film (Hyperfilm  $^3$ H, Amersham). For quantitative studies, all tissue sections are processed in parallel and the radioactive slides exposed to film with calibrated  $^{14}$ C-standards (ARC, Inc, St. Louis, MO). This robust technique can be used with both slow passive frozen human brain tissue as well as flash-frozen material, although this is somewhat dependent upon the receptor of interest.

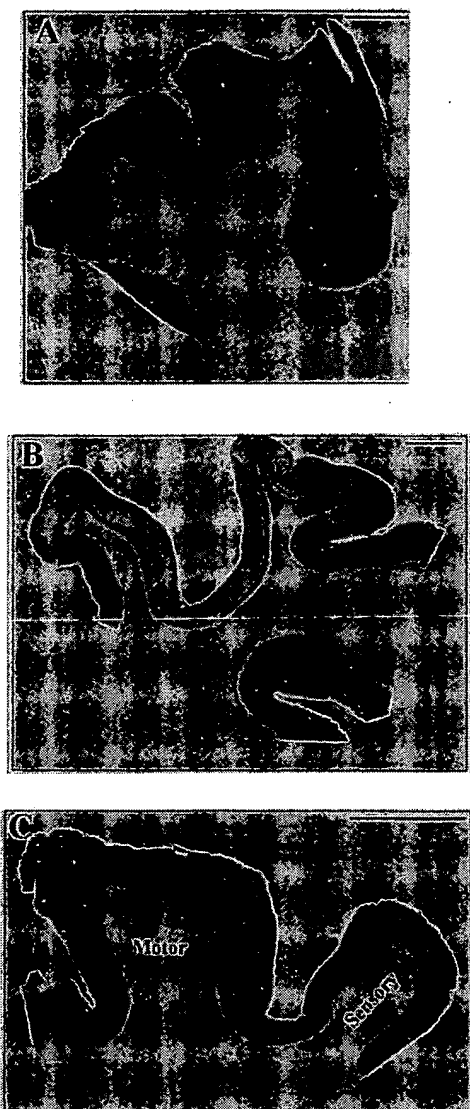


Fig. 6. Low power magnification of human brain cortex sections immunolabelled with antiserum selective for mGluR2. Sections are from the midregion of each cortical area. (A) Medial frontal gyrus (MFG). (B) Visual cortex (VC). (C) Sensorimotor cortex (SM). In A–C, mGluR2-LI is restricted exclusively to the gray matter (Phillips et al., 2000).

## 7. Screening for changes in gene expression using differential display technique

Recent advances in molecular biology techniques now facilitate the study of the etiology and pathophysiology underlying complex psychiatric and neurological disorders. Profiling of gene expression at the transcriptional level is a starting point in gaining insight into the function of known disease genes and allow the identification of novel genes and gene products that may be associated with a given disease process. As normal cell function is dictated by a highly regulated pattern of gene expression, any change in this homeostasis can lead to pathological states. The identification of genes that are abnormally expressed in as yet ill understood neuropsychiatric disorders, for example, is likely to open new windows for scientific study. Differential display is a PCR based method in which two sample populations i.e. normal versus pathological tissue can be compared. The technique allows high throughput screening and is both fast and reliable (Kozian and Kirschbaum, 1999; Liang and Pardee, 1992). Although DD-PCR techniques are relatively new they have already been successfully applied to address a number of neuroscience questions. These include studies of nerve cell regeneration following sciatic nerve injury (Namikawa et al., 2000), ontogeny (Joseph et al., 1994), learning and memory (Inokuchi et al., 1996) and changes in gene expression following treatment with cocaine and amphetamine (Douglass and Daoud, 1996).

We have used an indexing-based differential display technique to generate expression profiles from RNA obtained from post-mortem brain tissue. This improved DD-PCR technique (Mahadeva et al., 1998; Shaw-Smith et al., 2000) uses digestion of cDNAs with class II restriction enzymes (e.g. *Bbv*I), producing fragments with every combination of possible bases in the cohesive ends. Under stringent conditions, the specific ligations with perfectly complementary overhangs





Fig. 7. Localization of calbindin D<sub>28k</sub>-immunoreactivity within the human caudate-putamen. The heterogeneous distribution of immunoreactivity is consistent with the enrichment of calbindin D<sub>28k</sub> protein within the 'matrix' compartment compared to the 'striosome' compartment (arrows).

partitions the cDNA fragments into non-overlapping sub populations. Internal cDNA restriction fragments are exponentially amplified by adaptor primer PCR and visualized by non-denaturing polyacrylamide electrophoresis. This method has a number of advantages over previous techniques, especially with respect to reproducibility, particularly that intra-genic fragments are detected as opposed to 3' fragments. The fragments can be readily cloned and identified by comparison with database entries. In comparison to conventional DD-PCR methods (Liang and Pardee, 1992; Welsh et al., 1992), the technique has a higher specificity, i.e. a lower false positive rate and avoids redundancy between cDNA subsets.

In an initial study we compared the expression profiles of the prefrontal cortices of Alzheimer's disease and control cases and found robust changes in a number of mRNAs including GAP-43 and calcineurin (dramatically reduced in Alzheimer's disease samples) (Fig. 9). As GAP-43, a growth-associated protein, is expressed by cholinergic neurons (Augood et al., 1995), these data

are entirely consistent with a dysfunction in cholinergic signaling within Alzheimer's disease. Calcineurin is a protein-phosphatase that has been reported to be associated with the hyperphosphorylation of tau protein (Mayford and Kandel, 1999). Abnormally and/or hyperphosphorylated tau protein is a major component of neurofibrillary tangles and neuritic plaques, the most characteristic lesions in Alzheimer's disease to date.

Steps involved in optimizing the DD-PCR technique for human post-mortem brain tissue are as follows.

#### 7.1. RNA normalization

Although equivalent quantities (as measured by 260 nm absorbance) of total RNA are used for differential display, first strand cDNA concentrations should be further normalized prior to indexing, on the basis of semi-quantitative PCR amplification of housekeeping genes such as GAPDH or 18S ribosomal RNA. Some studies normalize to CREB mRNA (Ginsberg et al., 2000), although this may not be a universal marker.



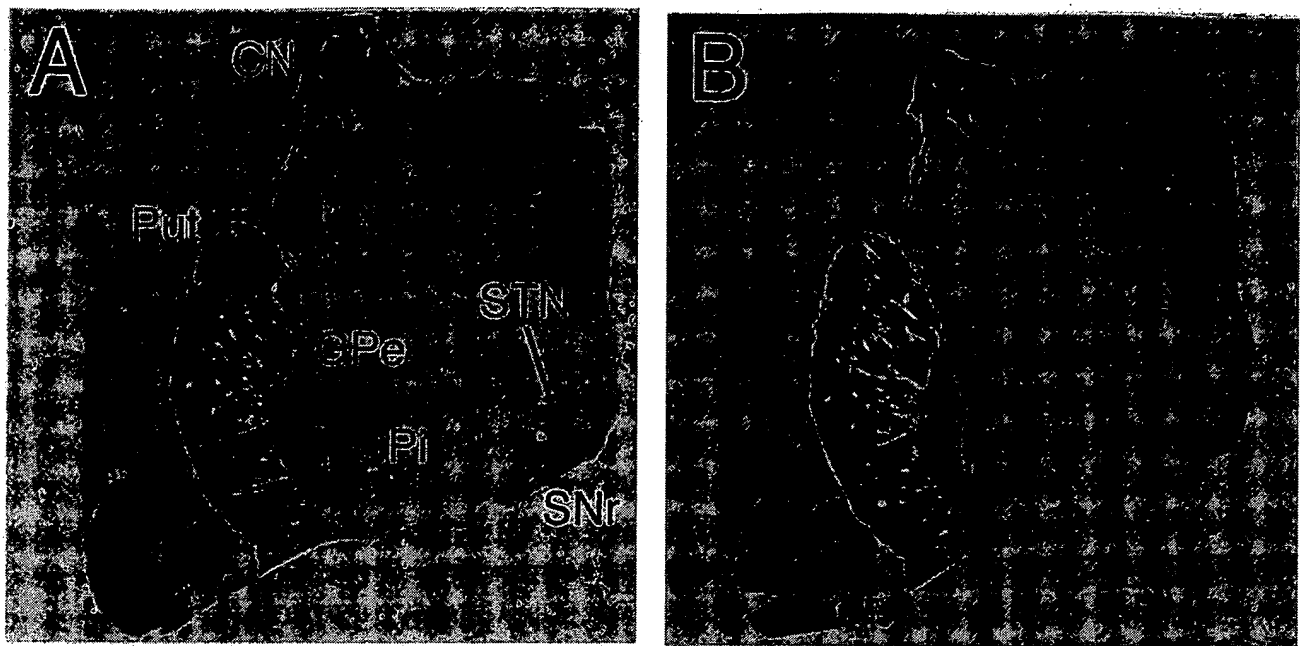


Fig. 8. Localization of DA D1-like (A) and D2-like (B) receptor binding sites in the human basal ganglia. Dopamine receptor binding sites were visualized using [ $^3$ H]-SCH23390 (D1-like) and [ $^3$ H]-YM 09151-2 (D2-like) as specific ligands. Note the enrichment of [ $^3$ H]-ligand within the caudate nucleus and putamen.

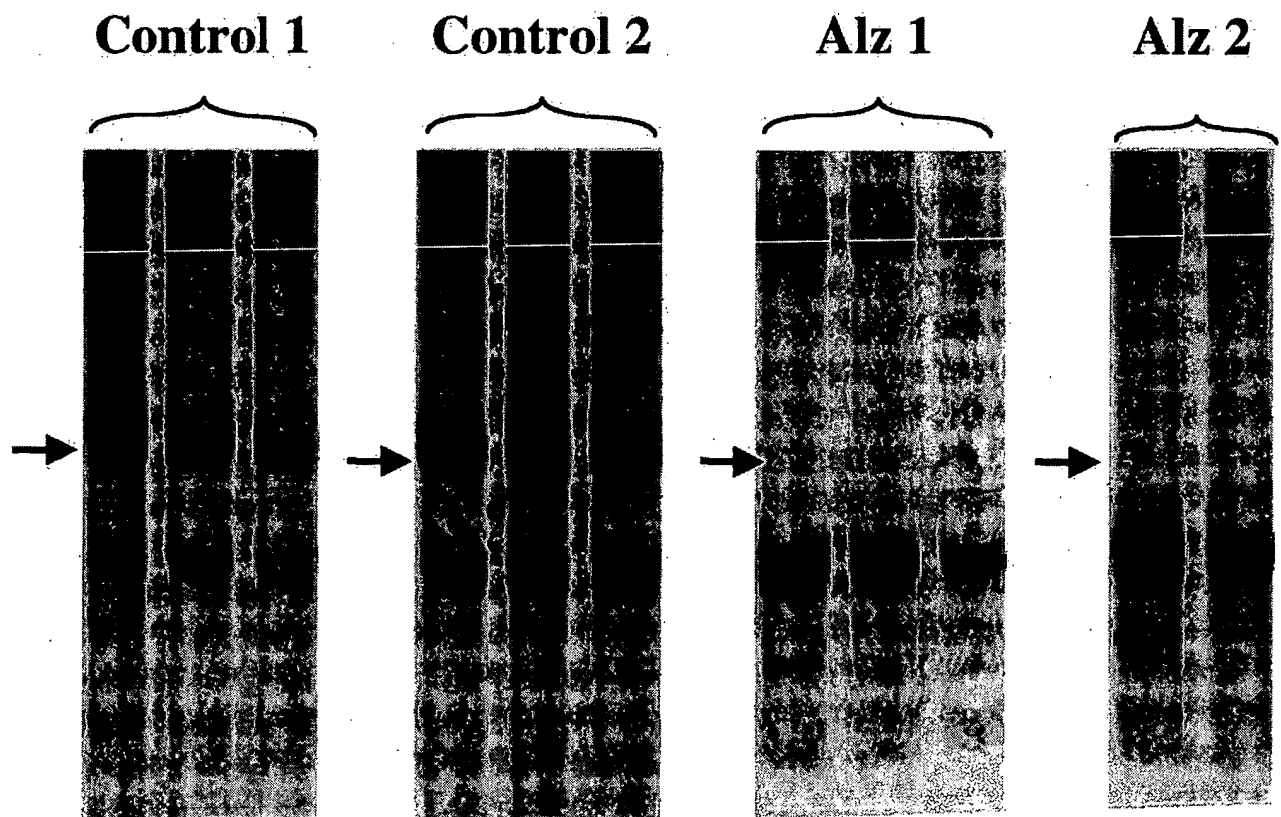


Fig. 9. Differential display gel — illustrating differences in gene expression between Alzheimer's and control individuals. Differentially expressed transcripts were identified by molecular indexing of *FocI* digests and subsequent non-denaturing PAGE analysis. Arrows indicate reduction in expression of GAP-43 in Alzheimer's samples.

### 7.2. Replicate samples

Replicate indexing reactions were performed and analyzed for each RNA sample to control for methodological problems (Fig. 9).

### 7.3. Controls

Each set of replicate indexing reactions includes a negative control, which represents a cDNA sample in the absence of reverse transcriptase. A further negative control included omission of sample cDNA to control for contaminating genomic DNA. Using this improved DD-PCR technique we have identified low abundance transcripts including G-protein coupled receptors which are assumed to be present at levels of less than 1 in 1000 molecules. These findings are in agreement with earlier studies which demonstrated that indexing is not prone to the elimination of low-abundance cDNAs (Mahadeva et al., 1998). Further, the high sensitivity of this DD-PCR method has enabled us to identify a number of novel differentially expressed mRNAs which currently (July 2000) do not correspond to any entries in available databases, including expressed sequence tags (EST) databases. Our results are highly reproducible both within and between individuals (Fig. 9) allowing minimization of false positives.

An advantage of this DD-PCR technique over earlier differential display protocols (Liang and Pardee, 1992; Livesey and Hunt, 1996; Welsh et al., 1992) is that relatively small amounts of total RNA are required, which is critical when only limited amounts of precious post-mortem tissue is available. As with all comparative differential screening techniques, one problem that plagues the technique is the percentage of false positives. Indexing-based DD-PCR minimizes this percentage (Mahadeva et al., 1998) by using mRNA-specific adaptor sets which facilitates the analysis of candidate genes which may have already been implicated in a disease process. Similar methodologies which have been used previously include traditional differential hybridization (Wietgreffe et al., 1985), subtractive cloning (Hara et al., 1991), subtractive hybridization (Duguid and Dinanuer, 1990) as well as the most recently developed techniques of serial analysis of gene expression (SAGE) (Velculescu et al., 1995). This SAGE technique is a sequence-based approach and is therefore time consuming and costly if automated sequencing and computational support is not readily available (Kozian and Kirschbaum, 1999).

## 8. Messenger RNA expression profiling and complex neurological disorders

The advent of mRNA expression profiling, facilitated

by the development of DNA arrays represents a major advance in functional genome analysis as alterations in cellular function caused by 'disease' mechanisms can impact directly on gene transcription. For example, oxidative stress has now been implicated in a number of genetic and idiopathic neurodegenerative disorders (Beal, 1996). 4-hydroxy-2,3-nonenal (HNE), a membrane lipid peroxidation product and biochemical marker of oxidative stress, has recently been shown to induce transcription and activation of AP-1 within these 'stressed' cortical neurons (Camandola et al., 2000). This activation involves a caspase/JNK transcriptional pathway thus providing insight into one mechanism by which HNE-mediated oxidative stress may ultimately lead to cell death. Further, by use of Affymetrix oligonucleotide arrays, Voehringer and colleagues have recently identified two proteins that are involved in disrupting normal mitochondrial function in an apoptosis-sensitive b-cell lymphoma (Lysa clonal cell line). Further, they also identified a pathway in the corresponding apoptosis-resistant cells (LYa clonal cell line) that may prevent mitochondrial uncoupling and induction of the caspase cascade (Voehringer et al., 2000). DNA arrays can also be used to accurately classify morphologically-identical cell types dependent upon their mRNA expression profile (Alizadeh et al., 2000; Khan et al., 1999). With regard to b-cell lymphomas, for example, this can have a dramatic impact on patient mortality via appropriate therapeutic intervention. Another recent study applied gene expression profiling to investigate the hypothalamus-pituitary-adrenal (HPA) axis to catalogue a large number of genes expressed in the HPA axis and led to the discovery of 200 novel genes (Hu et al., 2000). cDNA microarrays coupled with sub-cellular fractionation of polysomal mRNA have recently identified specific mRNAs coding for either secreted or membrane-bound proteins (Diehn et al., 2000). This sub-cellular fractionation approach allows one to investigate in more detail the ultrastructural physiology of the cell.

Thus, with the draft human genome sequencing project completed we are now able to screen for abnormal gene expression/transcriptional dysregulation in complex psychiatric and neurological disorders. Indeed, with the advent of mRNA expression profiling one can directly examine transcriptional dysregulation within the diseased brain (Lashkari et al., 1997; Lin et al., 2000; Luthi-Carter et al., 2000; Schena, 1996; Schena et al., 1996). There are currently numerous commercial products available for mRNA profiling including cDNA microarray membranes, cDNA microarray microscope slides and oligonucleotide Affymetrix GeneChips. All three approaches have their advantages and disadvantages, namely sensitivity versus cost although recent advances in linear amplification method-

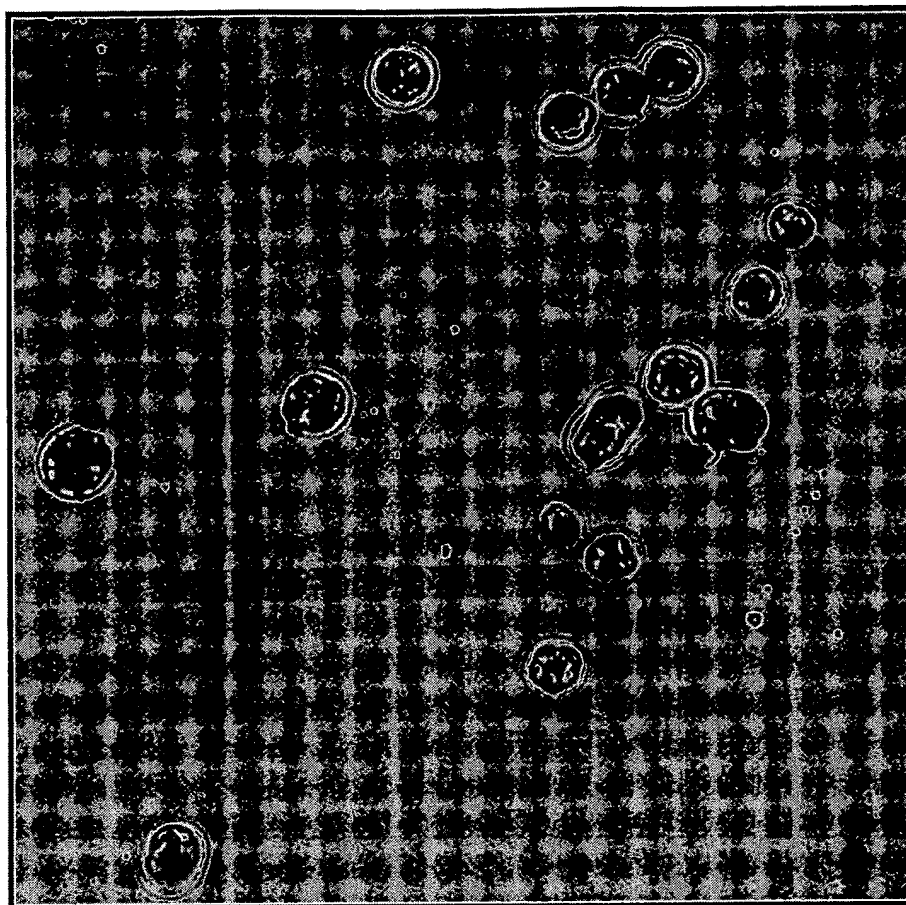


Fig. 10. Fourteen individual pigmented dopamine neurons laser-dissected from the substantia nigra pars compacta of a human post-mortem brain. The neurons have been laser-dissected from a rapid-frozen cryostat tissue section onto an Arcturus CapSure™ polymer cap.

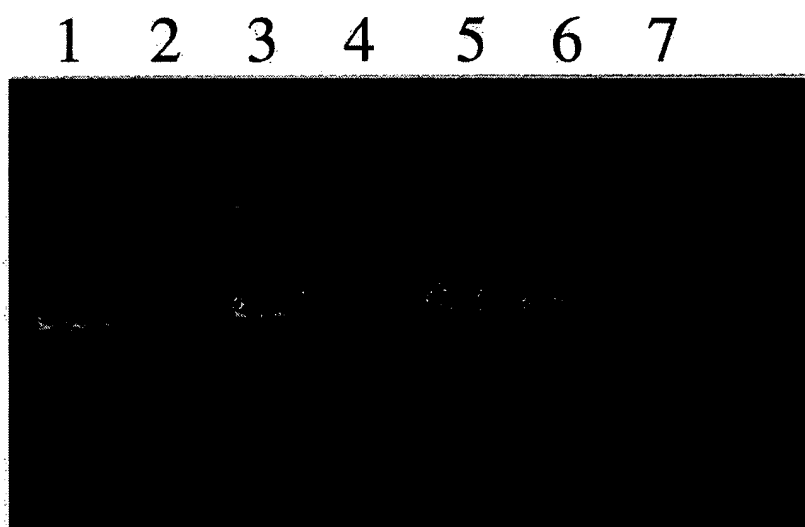


Fig. 11. U.V. transmission of an ethidium bromide-stained gel showing UCH-L1 PCR product amplified from total RNA extracted from LCM-dissected human DA nigral neurons. Lanes 1 and 3: b3688 + RT/b3229 + RT (expected product = 500 bp); Lanes 2 and 4: b3688 + RT/b3229 - RT (control for genomic DNA); Lanes 5 and 6: UCH-L1 template DNA (positive control); Lane 7: No DNA (negative control)

ologies may redress this balance (Ginsberg et al., 2000). Messenger RNA expression profiling studies are already yielding valuable data with regard to disease pathophysiology (Colantuoni et al., 2000) as well as aiding in patient diagnosis and treatment (Alizadeh et al., 2000; Khan et al., 1999). The greatest challenge associated with these mRNA expression-based screening techniques lies possibly in data analysis and data management (Ermolaeva et al., 1998) and (Eisen et al., 1998; Young, 2000).

### 9. Laser-capture microdissection (LCM)

With the advent of laser-capture microdissection (LCM) technology it is now possible to examine the

molecular basis of cellular function. For example, cellular resistance or vulnerability to excitotoxicity or disease can be investigated by micro-dissecting discrete cell populations under bright-field illumination from fresh-frozen cryostat tissue sections (Bonner et al., 1997; Emmert-Buck et al., 1996; Simone et al., 1998), followed by extracting total RNA and then examining the molecular signature of this cell population using mRNA expression profiling. The technique of LCM was initially developed within the cancer field (Emmert-Buck et al., 1996) to facilitate the segregation of benign and malignant cells and has only recently been applied to the study of the CNS (Luo et al., 1999). Essentially, a 3–8  $\mu\text{m}$  thick frozen/paraffin-embedded tissue section is rapidly processed for routine histology (Nissl, methyl green or H + E) or immunohistochemistry (Fend et al.,

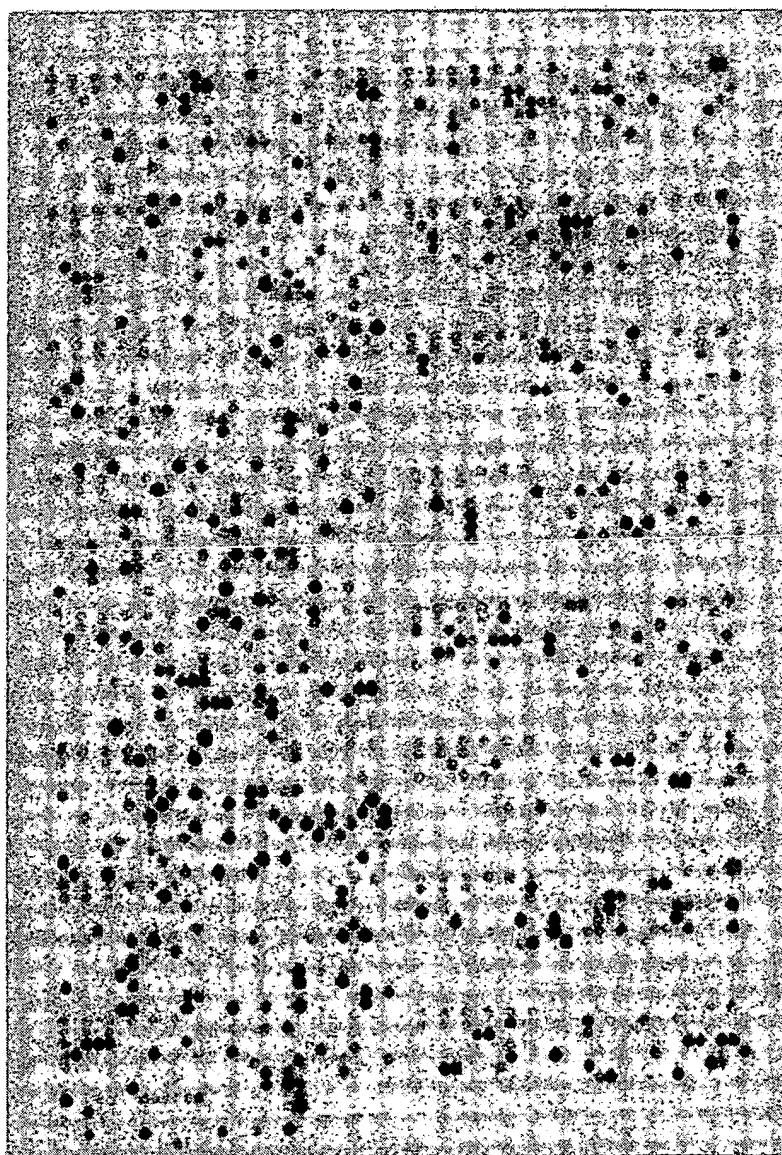


Fig. 12. Human 'Named Gene' microarray filter (Research Genetics GF-211) hybridized with a  $^{33}\text{P}$ -labelled DNA pool generated from LCM-dissected human neurons.

1999) using RNase-free reagents and then placed on an inverted microscope and overlaid with a ethylene vinyl acetate (EVA) transfer cap containing infrared absorbing dye. Neurons for laser-dissection are then selected and a laser pulse melts the EVA onto the targeted neuron where it solidifies. This LCM process can then be repeated hundreds of times with each cap. Selected EVA-impregnated neurons are finally harvested when the cap is removed from the tissue section: only the selected neurons are present on the EVA cap (Fig. 10). The cap is then fitted onto a sterile 0.5 ml Eppendorf tube containing RNA extraction buffer (guanidine isothiocyanate/ $\beta$ -mercaptoethanol) and the total RNA of the dissected neurons extracted.

The potential of this exciting new technique is enormous and has recently been used to study of the development and physiology of segregated populations of immune cells (Alizadeh et al., 2000), renal cells (Kohda et al., 2000) and benign and malignant prostatic epithelia cells (Ornstein et al., 2000). Methods for optimizing LCM protocols for RNA extraction and RT-PCR (Fig. 11) have now been established (Goldsworthy et al., 1999) and we and others are using these LCM methods coupled with cDNA microarray profiling (Fig. 12) to investigate neuronal vulnerability in PD and HD. These high-throughput array studies will add significantly to our understanding of neuronal vulnerability within these two degenerative diseases (Gibb, 1992; Kowall et al., 1987). Recently Ginsberg and colleagues (Ginsberg et al., 2000) used a similar approach to examine the differential mRNA profiles of neurofibrillary tangle (NFT)-positive and NFT-negative CA1 neurons in Alzheimer's disease: hybridization signal intensities were normalized to CREB mRNA. Of interest, they report a robust decrease in glutaredoxin mRNA (induced in response to oxidative stress) in the absence of a change in ubiquitin or microtubule-associated protein mRNAs. Such techniques will aid significantly the study of heterogeneous cell populations. Clearly, advances in mRNA and protein expression profiling (Banks et al., 1999; Lueking et al., 1999; Simone et al., 2000) will offer new opportunities for the study of neurological and neuropsychiatric disease.

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